

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
28 March 2002 (28.03.2002)

PCT

(10) International Publication Number
WO 02/24212 A1

- (51) International Patent Classification⁷: **A61K 35/78** - (74) Agent: SOHN, Chang, Kyu; 401, In-bong Bldg., 640-21, Yoksam-dong, Kangnam-gu, Seoul 135-080 (KR).
- (21) International Application Number: PCT/KR00/01034
- (22) International Filing Date:
25 September 2000 (25.09.2000)
- (25) Filing Language: Korean
- (26) Publication Language: English
- (71) Applicant (for all designated States except US): **ULTIMATE LIFE TECHNOLOGY CO., LTD.** [KR/KR]; 16-292, Jeongnung-dong, Seongbuk-gu, Seoul 136-100 (KR).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **LEE, Sang, Nam** [KR/KR]; 502-2, Shinsa-dong, Kangnam-gu, Seoul 135-120 (KR). **SONG, Yang, Hun** [KR/KR]; 105-1108, Samjeong Apt., Jeongrim-dong, Seo-gu, Taejon 302-230 (KR). **KIM, Soo, Kil** [KR/KR]; 102-1204, Jugong Apt., 765, Sanggae-dong, Nowon-gu, Seoul 139-200 (KR).
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- Published:
— with international search report
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: CHOLESTEROL IN BLOOD-LOWERING COMPOSITION AND ITS COMPLEX, AND METHOD FOR PREPARING THEM

(57) Abstract: The present invention relates to a composition and complex for reducing the cholesterol in the blood, particularly LDL cholesterol, and for curing the cholesteremia or some of heart diseases, as well as to a method for manufacturing those compositions and complex substances, wherein complexes or mixed compositions consisting of phyto-sterols, naturally occurring plant sterols, and tannins, as the main components, are used as pharmaceutical preparations, medicinal injections, food additives or the like to effectuate innovative reduction in the cholesterol value in the blood. Particularly, the complexes and mixed compositions according to the invention have the merit of convenience in that direct addition to the foodstuffs can produce the action of reducing the cholesterol value without doing any harm on human body.

WO 02/24212 A1

CHOLESTEROL IN BLOOD - LOWERING COMPOSITION AND ITS COMPLEX, AND METHOD FOR PREPARING THEM

5

FIELD OF THE INVENTION

The present invention relates to the composition and the complex for reducing the cholesterol in the blood, particularly LDL cholesterol, and for curing the cholesteremia or some of heart diseases, as well as to the method for manufacturing those compositions and complex substances, wherein complexes or mixed compositions consisting of phyto-sterols, naturally occurring plant sterols and tannins, as the main components, are used as pharmaceutical preparations, medicinal injections, food additives or the like.

15

BACKGROUND OF THE INVENTION

The cholesterol is an essential substance in human body, which plays a role in forming cell membrane, steroid hormones or the like. When the in-blood concentration of cholesterol, particularly LDL cholesterol or the lipid is maintained at excessively high level, however, there is a high possibility of heart diseases or arterial sclerosis, which can lead to death or disability.

20

As one of the methods for reducing the in-blood level of cholesterol, there is a proposed method wherein consumption of foodstuffs rich in the cholesterol or saturated aliphatic acids is suppressed, body weight is cut down and exercise amount is increased. In many cases, however, this method is neither so effective nor so easy to

25

follow. Accordingly, as a more concrete approach, a method for reducing the absorption of cholesterol in intestine tract is proposed, for which method some therapeutic agents and food additives are developed and used. However, this method is associated with some side effects and the efficacy is not so excellent as well.

5

On the other hand, in relevant studies, it has been repeatedly reported that the plant sterols are effective in controlling the cholesterol in the blood substantially without causing harmful effects (Reference: J. Nutr., 107, 2011 (1977)). Specially, the beta-cytosterol, which is advantageously acquired from bean or rice in easy ways and which turns similar to the cholesterol in structure when the ethyl group at the 24-th carbon is removed, has been confirmed to interfere with the cholesterol absorption in the human body through a competitive reaction (Reference: Atherosclerosis, 28, 325, (1977)).

15

Naturally occurring plant sterols (phytosterols) have the same skeleton in the structure, only slightly differing in side chains. The plant sterols taken-in together with foodstuffs are mostly either beta-cytosterols or campysterols, some of which are absorbed in the intestine and then discharged outside the body, so that even an over-intake causes no side effect on the human body due to only a minor part of it remaining in the body. Further, beside the plant sterols, their analogous products, i.e. stanol as hydrogenated compounds and their fatty acid ester compounds were also developed as the products for reducing in-blood cholesterol (Reference: J. Lipid Res., 20, 646, (1979); J. Nutr., 107, 1139(1977)), and recently the margarine added with the ester compounds of beta-cytostaneol was reported to reduce the cholesterol level in the blood (Reference: WO/19640). However, in spite of the efficacy of those developed products, the phyto-sterols have, in fact, the drawback that only an extremely limited amount of phyto-sterols is absorbed in micelle of the small intestines. Consequently,

20

25

difficulty arises that an excessive amount should be taken in order to realize a practical effect. Therefore, there is an urgent need for the development of new products that can reduce the cholesterol level more effectively while overcoming the afore-mentioned drawbacks.

5

SUMMARY OF THE INVENTION

In order to achieve the above-described object, the complex for reducing the in-blood cholesterol according to the present invention comprises, as the principal components, natural plant sterol ("phyto-sterol") and tannin at the weight ratio of 10:1 to 1:10.

The present inventors have confirmed that the phyto-sterol/tannin complexes manufactured by mixing naturally occurring plant sterols as harmless safe substances, and tannins have an unexpected synergic effect in reducing the in-blood cholesterol and so completed the present invention based on that finding.

As representative examples of phytosterols, beta-cytosterols, campy-sterols, stigma sterols, fuco-sterols, Brassica sterols may be mentioned. One of those sterols or a mixture of two or more may be used as the principal component for the products according to the invention.

The tannins as described above are generally included in the stems, leaves, roots of plants or the like, and also in the fruits and vegetables including unripe ones, as well as in the seeds of plants. In recent years, it was found that the tannin compounds have various physiological activities, which include, for example, the action of improving the blood circulation, anti-oxidation and other various

physiological activations.

The tannins may be classified as

(1) catechins and their derivatives,

5 (2) leucoanthocyanins,

(3) anthocyanidins,

(4) chlorogenic acids, which all belong to polyphenolic acids with phenol group(s).

10 As one component of the complexes according to the invention, the tannin compounds as described above can be used as a single compound or in the form of mixture of two or more compounds.

As the representative example of the catechins, there can be mentioned
15 catechin, which have the carbon skeletons of $C_6-C_3-C_6$, i.e. a structural characteristic common to the flavonoids, anthocyanins and the like. Under these tannins, catechins (trans-form), epi-catechin (cis-form), 3-galloyl epi-catechins and 3-galloyl gallocatechins are included.

20 Typical leucoanthocyanins may include leucoanthocyanidin, luteolinidin and the like.

The anthocyanidins have the basic structure of 3,5,7-trihydroxy flavylum chlorides. The anthocyanidin includes pelargonidin, cyaniding and delphinidin as the
25 representative examples and further includes hirsutidin (7-o-methylmalvidin), aurantinidin (8-hydroxy pelargonidin), apigeninidin (3-deoxy pelargonidin), petunidin 3,5-diglucoside, malvidin 3,5-diglucoside, peonidin 3-menoglucoside, malvin 3-

monoglucoside, cyanidin 3-galactoside, cyanidin 7-arabinoside, pelargonidin 3-monoglucoside, pelargonidin 3-galactoside, cyanidin 3-ramnosyl glucoside, cyanidin 3,5-diglucoside, cyanidin 3-diglucoside, cyanidin 3-lutioside and the like.

5 The representative examples of the above-described polyphenolic acids include chlorogenic acids, caffeine acid, phenyl caffeate, cinnamic acid, gallic acid and the tannic acid that is now known as a complex mixture of various compounds.

10 As is shown, the tannins have a variety of distributions and contents and they can be relatively easily extracted to give certain desired tannin.

15 The content ratio between phyto-sterol and tannin as the principal components of the complex according to the invention lies between 10:1 and 1:10 and more preferably between 4:6 and 6:4, each ratio being by weight, as mentioned earlier.

 If need be, other known components beside the above principal components may be added to promote the effect of the invention.

20 The inventors of the present invention have found that the phyto-sterol and tannin even in form of simple mixed composition, not in the form of a complex, has the synergetic effect. Accordingly, the present invention relates to a mixed composition comprising, as the principal components, phyto-sterol and tannin at the weight ratio of 10:1 to 1:10 for reducing the cholesterol in the blood.

25 However, the complex of phyto-sterol and tannin which is obtained by dissolving the phyto-sterol and tannin together in a solvent and then heat-treating the dissolved mixture at a defined condition further increases the synergic effect of mixing

the two ingredients.

Accordingly, the present invention relates to a method for manufacturing a complex of phyto-sterol/tannin as well.

5

The method for manufacturing the complex for reducing the in-blood cholesterol according to the present invention comprises dissolving the dried phyto-sterol and tannin in a solvent under an acidic condition, subjecting the dissolved mixture to reaction at a temperature from 20 to 100°C, removing the remaining
10 solvent from the product and finally subjecting the crude product to drying.

In particular, the manufacturing method comprises the steps of:

- (A) drying phyto-sterol and tannin separately at a normal temperature or near normal temperature;
- 15 (B) dissolving the dried phyto-sterol and tannin at the weight ratio of 10:1 to 1:10 in a solvent and stirring the dissolved mixture to provide a uniform solution;
- (C) heating the resulting solution at a temperature of 60 to 90°C for the period of 3 to 100 minutes and cooling the resulting solution
20 to a normal or near-normal temperature;
- (D) acidifying the solution by adding a weak solution, stirring the solution and heating the solution at a temperature of 60 to 90°C for the period of 3 to 100 minutes; and
- (E) removing the remaining solvent and drying the produced product.

25

More preferably, the temperature in the above steps (C) and (D) is about 70°C and the heating period is 5 to 30 minutes.

As the solvent used in the step (B), the water, ethanol or a mixture of water and ethanol may be appropriate.

5 As the weak solution for the step (D), preferably an edible acidic solution and most preferably vinegar from fruit may be used.

The complex and mixed composition according to the invention, as it is, or by adding other necessary ingredients, may be prepared into pharmaceutical formulations, medicinal injections, food additives or the like for reducing the cholesterol level in the blood.

For example, a pharmaceutical formulation may be prepared by adding some vehicle to the above-described complex as the active ingredient. A medicinal injection may be prepared by dissolving the above complex as the active ingredient in distilled water or saline solution as the solvent. Particularly, for the case of food additives that are added to the foodstuffs with high cholesterol content, powder of the complex is simply added to the raw material of foodstuffs and finished foodstuffs.

20 There is no upper limit to daily dosage of the complex because administering an excessive amount is not particularly harmful for a human body. However, a daily dosage of less than 10g per day for an adult with body weight of 60 kg is particularly preferable.

25 Therefore, the present invention relates to the agents for reducing cholesterol, injections for reducing cholesterol, food additives for reducing cholesterol, each containing the above-described complexes or mixed compositions as the active

ingredients, and various foodstuffs containing the above complexes.

The method for preparing the above agents, injections and foodstuffs is the same as the method for preparing ordinary medicinal agents or injections based on the medicinal active components, and therefore is no more described. For the case of manufacturing foodstuffs containing the present complexes or mixed compositions, addition can be made in a variety of ways only under the condition of not damaging the present main components. In other words, when an excessive heat is applied in the course of manufacturing a foodstuff, the addition is conducted at the unheated final stage, while an excessive heat is not applied in the course of manufacturing foodstuff, the complex material is added together with the raw material before processing. The foodstuffs are not restricted, as far as the form is concerned. For instance, the foodstuffs in liquid form such as beverage or the foodstuffs in solid form such as meat for hamburger can be covered.

Accordingly, the present invention relates to a method for reducing the in-blood cholesterol content and suppressing the cholesterol absorption by using the complex or mixed composition of interest in medicinal agents, medicinal injections, foodstuff additives or the like. Such a method includes the cure of high cholesterol symptoms and some of heart diseases.

While the present invention is described in more detail below along some examples, they are intended only for illustrative purpose and so not to restrict the scope of the invention.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Example 1: Manufacture of the complex of beta-cytosterol and catechin

200 mg of beta-cytosterol, a kind of phyto sterol, and 280 mg of catechin, a kind of tannin, were well mixed and dried at normal temperature for 30 minutes. To the resulting mixture, there was added 50 mg of aqueous ethanol solution, and the mixture
 5 was agitated for 1 hour to provide a homogenous solution. The solution was heated at 70°C for about 20 minutes. The resulting substance was cooled to the normal temperature over 30 minutes and then acidified by adding 10 ml of fruit vinegar. The acidified solution was further agitated at the normal temperature for 15 minutes and then heated at 70°C for about 5 minutes. The excessive solvent was removed, and the
 10 formed product was dried and filtered through a 20 mesh-sieve to produce a uniform product.

Example 2: Animal test for the complex

30 Sprague-Dawley rats with the age of 4 weeks were normalized for a week
 15 under the laboratory rearing condition and divided into 4 groups. The feed that was added with 1% of cholesterol or 0.5% of cholic acid was supplied to the rats for a week to increase the cholesterol value in the animals' blood. Subsequently, the feed, to which the complex of the invention was added at the concentration of 0.5%, 1% or 3%, was supplied, while the total cholesterol values, LDL-cholesterol values and HDL-
 20 cholesterol values were measured over the time period. The results are listed in Table 1.

[Table 1]

Group	Condition	Total cholesterol value (Reduction rate)	LDL	HDL
Group 1 (Control group)	1% cholesterol + 0.5% cholic acid	254	172	79

Group 2	1%cholesterol+0.5% cholic acid+0.5% complex	209 (18%)	135	72
Group 3	1% cholesterol+0.5% cholic acid+1% complex	186 (27%)	119	70
Group 4	1% cholesterol+0.5% cholic acid+3% complex	169 (34%)	91	71

(The listed data represent the result after 2 weeks for the groups 2 and 3, and the result after 5 days for the group 4).

As can be seen from the above table 1, the effect of reducing the cholesterol in the blood, exhibited by the complex according to the invention, was very excellent. It is also understood that at the low concentration of the complex in Groups 2 and 3, the effect showed up so slowly that the total cholesterol values were reduced 18% and 27% respectively after two weeks, while at the higher complex concentration of 3% in Group 4, reduction of 34% in the cholesterol value was achieved only in 5 days.

Accordingly, it is confirmed that the complex substance of the present invention reduces the total cholesterol number in the blood as well as the LDL-cholesterol without influencing the absorption of favorable HDL-cholesterol to attain a satisfactory result.

Industrial Applicability

As described above, as the complexes according to the invention are effective in curing high cholesterol symptoms and in preventing heart diseases and hypertension, they can be used not only in medicinal preparations or injections but also can be used in

manufacturing health-oriented functional foodstuffs by simply incorporating in other foodstuffs and beverages.

WHAT IS CLAIMED IS:

1. Complex for reducing the cholesterol in the blood, characterized in comprising,
as the principal components, natural plant sterol ("phyto-sterol") and tannin at the
5 weight ratio of 10:1 to 1:10.

2. The complex for reducing the cholesterol in the blood according to Claim 1, in
which said phytosterol is one or a mixture of two or more selected from beta-
cytosterols, campy-sterols, stigma sterols, fuco-sterols and Brassica sterols;

10 Said tannin is one or a mixture of two or more selected from compounds
belonging to the below:

(1) catechins and their derivatives,

(2) leucoanthocyanins,

15 (3) anthocyanidins,

(4) chlorogenic acids, which all belong to polyphenolic acids with phenol
group(s).

3. The complex for reducing the cholesterol in the blood according to Claim 2, in
20 which

as said catechins and their derivatives, there are catechins (trans-form), epi-
catechin (cis-form), 3-galloyl epi-catechins, 3-galloyl gallocatechins and the like;

as said leucoanthocyanins, there are leucoanthocyanidin, luteolinidin and the
25 like;

as said anthocyanidins, there are anthocyanidin, pelargonidin, cyanidin,
delphinidin, hirsutidin (7-o-methylmalvidin), aurantinidin (8-hydroxy pelargonidin),

apigeninidin (3-deoxy pelargonidin), petunidin 3,5-diglucoside, malvidin 3,5-diglucoside, peonidin 3-menoglucoside, malvin 3-monoglucoside, cyanidin 3-galactoside, cyanidin 7-arabinoside, pelargonidin 3-monoglucoside, pelargonidin 3-galactoside, cyanidin 3-ramnosyl glucoside, cyanidin 3,5-diglucoside, cyanidin 3-diglucoside, cyanidin 3-lutioside and the like;

as said polyphenolic acids, there are chlorogenic acids, caffeine acid, phenyl caffeate, cinnamic acid, gallic acid, the tannic acid and the like.

4. Composition for reducing the cholesterol in the blood, characterized in comprising, as the principal components, natural plant sterol ("phyto-sterol") and tannin at the weight ratio of 10:1 to 1:10.

5. Medicinal agents, medicinal injections or food additives containing, as the active ingredients, the composites or the compositions for reducing the cholesterol in the blood according to one of Claims 1 to 4.

6. Foodstuffs containing, as the active ingredients, the composites or the compositions for reducing the cholesterol in the blood according to one of Claims 1 to 4.

7. Method for manufacturing the phyto-sterol and tannin complex for reducing the in-blood cholesterol, characterized in comprising dissolving the dried phyto-sterol and tannin in a solvent under an acidic condition, subjecting the dissolved mixture to reaction at a temperature from 20 to 100°C, removing the remaining solvent from the product and finally subjecting the crude product to drying.

8. Method for manufacturing the phyto-sterol and tannin complex for reducing the in-blood cholesterol according to Claim 7, in which the manufacturing method

comprises the steps of:

(A)drying phyto-sterol and tannin separately at a normal temperature or near normal temperature;

5 (B)dissolving the dried phyto-sterol and tannin at the weight ratio of 10:1 to 1:10 in a solvent and stirring the dissolved mixture to provide a uniform solution;

(C)heating the resulting solution at a temperature of 60 to 90°C for the period of 3 to 100 minutes and cooling the resulting solution to a normal or near-normal temperature;

10

(D)acidifying the solution by adding a weak solution, stirring the solution and heating the solution at a temperature of 60 to 90°C for the period of 3 to 100 minutes; and

(E) removing the remaining solvent and drying the produced product.

15

9. Method for manufacturing the phyto-sterol and tannin complex for reducing the in-blood cholesterol according to Claim 8, in which the temperature in the above steps (C) and (D) is about 70°C and the heating period is 5 to 30 minutes;

the solvent used in the step (B) is water, ethanol, or a mixture of water and ethanol; and

20

the weak acidic solution used in the step (D) is vinegar from fruit.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR00/01034

A. CLASSIFICATION OF SUBJECT MATTER**IPC7 A61K 35/78**

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Korean Patents and applications for inventions since 1975

Korean Utility models and applications for utility models since 1975

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KR 2000-0019718, 15 APR 2000 claim 1-5	1-3, 5-6
A	KR 1994-0025579, 8 DEC 1994 claim 1-3	1-3, 5-6
A	PHARMACOL. RES., vol. 35, no. 6, pp. 505-512 (1997) see the whole document	1-3, 5-6
A	AM. J. MED., vol. 107, no. 6, pp. 588-594 (1999) see the whole document	1-3, 5-6
A	LIPIDS, vol. 27, no. 3, pp. 181-186 (1992) see the whole document	1-3,
A	AGRIC. BIOL. CHEM., vol. 54, no. 1, 171-175(1990) see the whole document	1-3,



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

29 DECEMBER 2000 (29.12.2000)

Date of mailing of the international search report

29 DECEMBER 2000 (29.12.2000)

Name and mailing address of the ISA/KR

Korean Industrial Property Office
Government Complex-Taejon, Dunsan-dong, So-ku, Taejon
Metropolitan City 302-701, Republic of Korea

Facsimile No. 82-42-472-7140

Authorized officer

YOU, In Kyoung

Telephone No. 82-42-481-5610



INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR00/01034

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
KR 2000- 0019718	15. 04. 2000	NONE	-
KR 1994- 0025579	08. 12. 1994	NONE	